Fluorescence Quenching by 4-(2-hydroxyethyl)-1-piperazineethanesulfonic

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Abstract – **Quenching in fluorescence spectroscopy is crucial in numerous applications and can reveal important information about biochemical systems. The purpose of this study was to investigate if the 4-(2-hydroxyethyl)-1- piperazineethanesulfonic acid (HEPES) has a quenching effect and draw a correlation between reduction potential and quenching. Although for C343 the quenching effect was negligible, HEPES decreased the fluorescence responses of the BODIPY dyes significantly. A direct correlation between quenching rate and reduction potential was established.**

I. INTRODUCTION

In fluorescence spectroscopy, fluorescence intensity of fluorophores dictate the applications and efficiency of fluorophores[1]. Quenching, any decrease in fluorescence intensity, can reveal information about biochemical systems and the electron transfers among donor-acceptor systems [2]. There are numerous applications of quenching: proteins, membranes, fluorescence probes research and indication of DNA hybridization and potassium ions and dye sensitized solar cells [3] Quenching is quantitatively measured using the Stern-Volmer equation[4].

The reduction potential, or how easily a molecule can gain electrons, can help predict quenching rates in cases where quenching occurs due to electron transfer.

Anilines, piperidines, pyrazines, and morpholines, molecules that are similar to Good's buffers, have cause quenching among various fluorophores [5]. Good's buffers were developed during 1966–1980 specifically for biochemical and biological studies. A specific Good's buffer is 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) which is often used environmental, analytical and biological [6]. This paper will focus on how the HEPES buffer affects the fluorescence intensity of three different fluorophores.

The three fluorophores used in this study are Coumarin 343 Ethanolamine Amide Derivative (C343), BODPIY FL, and BODIPY FL-iso-chloride. The excitation and emission wavelengths and the electrochemical properties are included for each fluorophore in Table 1. C343 is often used in laser dyes, synthesizing electrodes and development of chemosensors and polymers [7]. BODIPY FL is used in the separation of glycosphingolipids, monitoring protein synthesis and in fluorescent probes [8]. BODIPY FL Iso-Chloride is a newly synthesized fluorophore and was used in this study to analyze its photophysical properties. These two commonly used classes of fluorophores have many wide applications and therefore were chosen to be studied.

Currently, it is unclear whether there is a quenching effect of fluorophores caused by buffers. The purpose of this studyis to determine if the HEPES buffer has a quenching effect on three fluorophores, C343, BODIPY FL and BODIPY FL Iso-Chloride, and to draw a correlation between reduction potential and quenching.

II. METHODS

A 500 mM stock solution of HEPES was prepared from Sigma Life Science (Lot #SLBK 1535V). Dilutions ranging from 5mM to 400mM HEPES were created. 1M KOH and 11M HCl were used to adjust the pH to 8.00±0.02.

Three sets of titrations were done: each fluorophore with increasing concentrations of HEPES. The instruments used were an Agilent Technologies Carian Cary 100 UV-Vis Spectrophotometer and a QuantaMaster 40 Photon Techonologies. To begin the titration, 1.5 mL of 0mM HEPES buffer was added into two cuvettes. One was placed in the reference slot and the other in the sample slot of the UV-Vis spectrophotometer. The sample cuvette was then removed and placed into the fluorimeter. The cuvette was rinsed three times with water and once with acetone. N_2 gas was used to dry the cuvette. This was repeated for all the remaining concentrations of HEPES and then for each fluorophore.

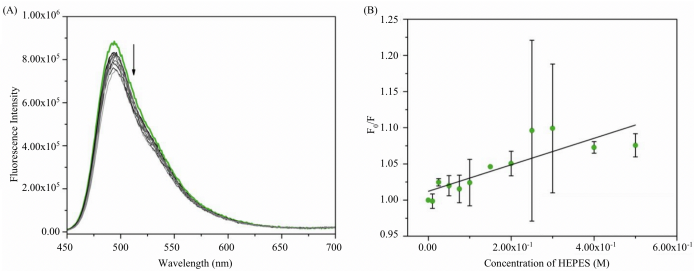

Figure 1. Emission Spectra from Quantamaster 40 Photon Technologies (A) and Stern-Volmer Plot (B) of C343 in presence of increasing concentration of HEPES buffer at 25 °C. Y-error bars in Figure 1B show standard deviation.

III. RESULTS AND DISCUSSION

Three titrations with various concentrations of the HEPES buffer and the three different fluorophores were performed to analyze the effect of the HEPES buffer on fluorescence intensity.

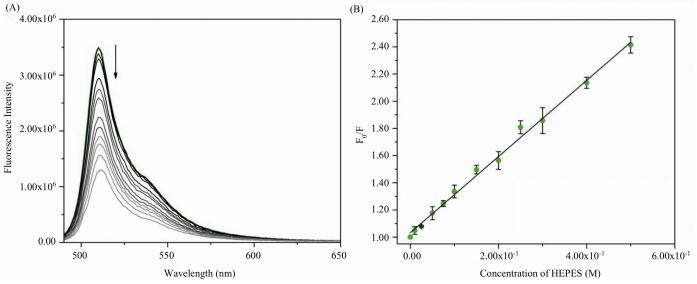

Figure 2. Emission Spectra from Quantamaster 40 Photon Technologies (A) and Stern-Volmer Plot (B) of C343 in presence of increasing concentration of HEPES buffer at 25 °C. Y-error bars in Figure 2B show standard deviation.

The emission spectra for the C343 titration shows minimum gaps between each trace and it seems as if the lines are on top of each other (Figure 2A). This indicates as

the concentrations of HEPES buffer increases, the fluorescence intensities do not change significantly, signifying the HEPES buffer does not have a quenching effect. The Stern-Volmer Plot shows a very small slope of 0.18301 indicating a negligible quenching rate (Figure 2B) [9]. (Table 1)

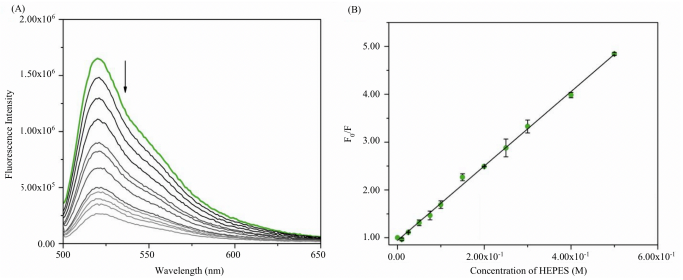

Figure 3. Emission Spectra from Quantamaster 40 Photon Technologies (A) and Stern-Volmer Plot (B) of BODIPY FL Iso-Chloride in presence of increasing concentration of HEPES buffer at 25 °C. Y-error bars in Figure 3B show standard deviation.

Properties	Fluorophore		
	C343	***BODIPY FL***	***BODIPY FL Iso-Chloride***
Excitation λ	446 nm	503 nm	500 nm
Emission λ	462 nm	512 nm	521 nm
Reduction Potential	-1.652V vs. SCE	-1.004V vs. SCE	-0.832V vs. SCE

Table 1.

In the emission spectra for BODIPY FL, there are larger gaps in the fluorescent intensity of the traces as concentrations of HEPES increase (Figure 3A). The Stern-Volmer Plot has a slope of 2.80248 (Figure 3B) [11].

The reduction potential of BODIPY FL is -1.004 V vs. SCE which is higher than C343 showing that more easily reduced fluorophores result in greater amounts of quenching (Table 1).

The emission spectra for the BODIPY FL-iso-chloride has very large gaps between each trace showing that as concentrations of the HEPES buffer increase, they have greater decreasing effects on fluorescence intensity (Figure 3A). The Stern-Volmer Plot shows the highest slope of 7.78364 for all three titrations (Figure 3B) . BODIPY FL-Iso-Chloride had the highest reduction potential of -0.832 V vs. SCE—showing that it is easily reduced-- and also the greatest amount of quenching [10].

IV. RESULTS AND DISCUSSION

The effect of increasing concentrations of the HEPES buffer in presence of fluorophores with different electrochemical properties were studied. The fluorophores that are easily reduced showed higher quenching effect by the HEPES buffer. Therefore, it can be established that there is a direct correlation between the quenching by the HEPES buffer and reduction potential. Thus, fluorophores that are easily reduced (low reduction potentials) should always be investigated to see if a quencher can induce significant quenching. C343 had a negligible amount of quenching, however BODIPY FL and BODIPY FL-Iso Chloride had significant amounts of quenching. Although the HEPES is very commonly employed, it may not be a good buffer for photophysical characterization of easily reduced fluorescent dyes because of its ability to quench fluorescence intensity.

Future investigations should include studying more aqueous buffers and fluorophores to determine if this correlation between reduction potential and quenching rate is consistent [3]. Other fluorophores that should be investigated include Alexa dyes, eFluor dyes, oxazines, and Nile blue dyes[12]. In addition, using time resolved fluorescence spectroscopy and analyzing absorption spectra can reveal vital information about the mechanism of quenching which is crucial in determining its applications like in dye sensitized solar cells and fluorescent probes [13].

V. ACKNOWLEDGEMENTS

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VI. REFERENCES

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